Determining the presence of Iron-Related Bacteria in water

Used in oil, gas and petrochemical industries, air industries, food industries, water and waste water, etc

Iron plays an important role in energy metabolism, so there is a lot of biological competition for it. Iron-Related Bacteria are a group of bacteria that carry out their metabolism by receiving carbon from carbon dioxide in the air and obtaining the energy they need from soluble iron. These bacteria cause the accumulation of iron in the parts where there is accumulation of microbial growth (biofilm-slime). Usually, this accumulation of iron is in the form of ferric iron crystals in the form of oxides, hydroxides and sometimes carbonates. This process creates a red-brown color. The result of the activity of IRB bacteria can cause corrosion in the facilities of various industries such as oil, gas, water and waste water, as well as causing odor, bad taste and red color in drinking water.

It is difficult to identify and count Iron-Related Bacteria due to the diversity of its bacterial groups (iron-reducing bacteria and iron-oxidizing bacteria). MicrobCheck™ IRB test kit is able to identify and estimate the relative population of both groups.

The MicrobCheck™ IRB test kit is designed as a 50 ml falcon containing culture medium and a floating ball.

Manufacturer's Recommendation

Avoid contact with the inner wall of the falcon and perform the test under sterile conditions.

After opening the Falcon, place the door upside down, with the bottom facing the ground, on a clean surface.

Make sure to take the sample from the right place.

Note that IRB bacteria mainly grow on surfaces and not directly in running water. For this reason, if the sampling is done only from running water, the test can be associated with a false negative result despite the presence of relevant bacteria. In order for IRB bacteria to be released into the water, it is necessary to slightly change the environmental conditions. For example, water pumping should be changed or a mild chemical shock should be used. This shock can be done with a low dose of hypochlorite.

Test Method

Preparation

Collect at least 25 ml of samples.

Pour 19 ml of the sample into the falcon and close it.

Do not shake or rotate the falcon after the sample has been added. Let the ball float on the surface of the culture medium.

On the falcon, note the date and name of the specimen.

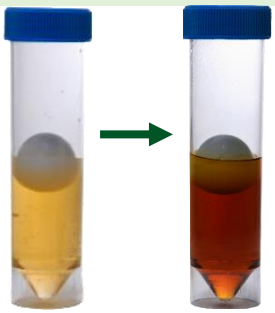
Incubation

Incubate the falcon at room temperature (21-25°C) away from sunlight.

View the sample daily for 8 days. Note the date of the first observed reaction.

If the reaction is negative, keep the sample until the fourteenth day and check it daily.

Presence / Absence



A pattern of positive results has been defined in the MicrobCheck™ IRB test kit, which can characterize the two main characteristics of IRB bacteria, i.e., whether they are aerobic or anaerobic.

Anaerobic IRB bacteria are characterized by the formation of brown-orange, gray or black deposits on the bottom of the falcon.

Aerobic IRB bacteria are characterized by the formation of a brown-orange layer around the floating sphere. In this case, a gas bubble or a layer of foam may be seen around the ball.

Note that in the condition that the water sample is saturated with gas, gas bubbles may form on the wall of the falcon. It should be noted that these gas bubbles are not a sign of bacterial activity. The only bubbles that can be seen around the floating ball are an indication of the activity of IRB bacteria.

A diverse population of aerobic and anaerobic IRB bacteria is associated with a change in the color of the culture medium to brown-orange along the entire length of the falcon, which indicates the presence of a combination of IRB with diverse aerobic needs, ranging from anaerobic to microaerophilic and aerobic.

Reaction Patterns

A Set of reactions can be seen in the MicrobCheck™ IRB test kit. In order to better see these reactions, it is better to examine the falcons in front of the light so that the growth pattern can be determined better.

It should be noted that each of the mentioned reactions is produced in a unique way by different strains. Therefore, it can be said that there is no special form of any of the mentioned reaction patterns because these patterns are controlled by the bacterial growth model.

Clouded Growth (CL): When there is a population of aerobic bacteria, the initial growth may be cloudy or fluffy. This growth is seen in gray color. If the falcon is slightly rotated, the clouds move to maintain the position in the falcon. Usually, the culture medium below the cloud area is darker and above it, brighter.

Brown Gel (BG): In this reaction, the growth in the bottom of the falcon occurs in the form of a jelly with a brown color. If the falcon is rotated or the gel is separated from the bottom of the falcon, it maintains its structure and position. The brown gel can occupy the entire inner cone of the falcon bottom. Usually, the culture medium above this gel is completely transparent and colorless. The volume of the brown gel increases at first and then decreases.

Brown cloudy (BC): Except when the population of IRB bacteria in the sample is high, the BC reaction is usually the second reaction and often occurs after CL, FO or RC. This reaction is seen as a dirty brown solution and may be accompanied by a brown ring around the ball.

Foam (FO): This reaction is easily recognized because the gas bubbles create a foamy ring around the ball. Sometimes the bubbles accumulate around more than 50% of the bottom surface of the ball. Sometimes gas bubbles accumulate on the walls of the falcon, but as long as they are not around the ball, it is not acceptable. In this pattern, the solution usually remains clear, but is accompanied by a change in color to yellow or yellow-green. Sometimes the bubbles may be covered with slime. In this case, the bubbles are seen with different colors such as brown, orange, yellow or gray. When slime is combined with foam, the structure of the foam hardens in such a way that it lifts the ball from the solution or sinks it below the surface of the solution. The foam pattern is different from the conditions in which bubbles are produced due to oxygen supersaturated conditions. Random bubbles usually disappear after 2 days. The foam growth pattern usually indicates a sample that has a large population of anaerobic bacteria and is also associated with a positive test in MicrobCheck™ SRB.

Red, Slightly Clouded (RC): The medium remains as a clear to dark brown solution. Usually, after observing BR around the ball, a BC cloud structure is also formed in the solution.

Brown Ring (BR): A ring of brown-red to dark brown slime is formed around the ball. The ring formed is usually tight and more than 3 mm wide. In some cases, the BR reaction can lock the ball into the falcon wall. In this case, when the falcon is reversed, the ball remains in place and the culture medium is placed on top of the ball.

Green Clouded (GC): The solution turns green. Although specific cloud or gel-like structures are not formed, cloud structures are observed in the culture medium. The slime ring does not form around the ball. The cloudiness of the solution gradually increases and the solution becomes dark green. The higher the amount of cloudy structures and the green color of the solution, the more likely the BR reaction will occur.

Blackened Liquid (BL): It is usually the second or third reaction to the first reaction. BL can be seen as large black areas on the cone as well as the walls of falcon. In this reaction, the solution remains clear.

Fuzzy: It is observed in less than 1% of the tests. If this result is obtained, it indicates that the water passed through an area where there was fungal activity. Villi are usually seen as a white to gray layer around and above the ball. The growth of these villi can lock the ball inside the wall of the falcon for some time. The solution usually remains clear, but globular masses may be seen. Over time, the solution also becomes cloudy, which is caused by large populations of fungal spores in the water.

BG, BL, GC and RC reactions can indicate hygiene risks.

Considering that IRB bacteria are present in slimes and an accumulation of bacteria, a chain of all kinds of reaction patterns can occur according to the nature of the bacteria in the sample.

Dominant population of bacteria	Reaction Pattern Signatures
BC - WB – BR	IRB bacteria along with carbonate deposition and the presence of some slime producing bacteria
CL - GC	A combination of IRB heterotrophic bacteria with the dominant majority of *Pseudomonas*
CL – BG	A combination of heterotrophic IRB bacteria with some enteric bacteria (probably *Enterobacter*)
CL – BC	A combination of heterotrophic bacteria
CL – BC – BR	A combination of heterotrophic bacteria with some slime producing bacteria
CL – FO	IRB bacteria with a combination of aerobic bacteria
CL - BC	Aerobic IRB bacteria with white carbonate deposition
FO – CL	Anaerobic bacteria along with some aerobic heterotrophic IRB bacteria
FO – CL - RC	Anaerobic bacteria along with some aerobic heterotrophic IRB bacteria and enteric bacteria (probably *Enterobacter*, *Citrobacter* and *Serratia*)
FO- CL – BC - BR	A combination of anaerobic, enteric bacteria and some IRB bacteria forming slime
FO – BR – BC	A combination of anaerobic bacteria and IRB together with aerobic slime forming bacteria
FO – GC	A combination of anaerobic and aerobic bacteria with a dominant population of *Pseudomonas*
FO – GC – BL	A combination of anaerobic bacteria, *pseudomonas* and enteric bacteria
GC	Dominant population of *Pseudomonas*
GC – BL	The dominant population of *Pseudomonas* along with some enteric IRB bacteria
RC – CL -BR	Dominant population of enteric bacteria

Estimation of Population and Aggression Level

The aggression level of IRB bacteria present in the water sample is determined based on the duration of incubation to observe the first reaction. Incubation time of 5 days or less is considered as high aggression level. Average aggression level defined between 5 and 8 days, and 8 to 10 days indicates moderately aggressive of IRB bacteria. Incubation time above 10 days is considered as background and normal. In the table below, the relationship between the incubation time and the degree of aggressivity and the relative number of IRB bacteria is described.

If the number of bacteria in the sample shows very aggressive or moderate aggressive, it is necessary to check the presence of *coliforms* to ensure that intestinal *coliform* bacteria are not present in the sample. It should be noted that if the test is performed for all *coliform* bacteria, it can be associated with a positive result because some of the bacteria that have led to the reaction are environmental enteric bacteria. If the reaction occurrence pattern includes GC, it is necessary to perform a test for the presence of fluorescent producing *Pseudomonas* bacteria.

Aggression Level	Time Lag (day)	Population (cfu/ml)
Very Aggressive	1	540,000
Very Aggressive	2	140,000
Very Aggressive	3	35,000
Very Aggressive	4	9,000
Very Aggressive	5	2,300
Moderately Aggressive	6	500
Moderately Aggressive	7	150
Moderately Aggressive	8	25
Normal Background	9	10 ≥

Quality Control of the MicrobCheck™ IRB Test Kit

To confirm the accuracy and performance of the MicrobCheck™ IRB test kit, the specified strains can be cultured and reaction patterns can be checked. After adding the bacterial suspension, do not shake the falcon and let it slowly enter the culture medium. Keep the inoculated falcons at 21-25 °C for 8 days and check the activity and reactions daily.

Some of the above tests may change over time from one reaction pattern to another. For example, after 5 to 8 days, *C. freundii* causes the ball to be locked in the wall of the falcon. It is necessary to perform the *E. coli* test at a temperature of 35 degrees Celsius.

Organism (ATCC)	Pattern
Citrobacter freundii (8090)	GC
Enterobacter aerogenes (13048)	BR
Pseudomonas aeruginosa (27853)	GC
Acinetobacter calcoaceticus (19606)	GC
Enterobacter cloacae (23355)	CL – BG
Proteus vulgaris (13315)	CL – BC
Klebsiella pneumoniae (13883)	RC – BC
Escherichia coli (25922)	FO

Best Time to Use

The expiration date of the kits is 6 months and it is necessary to store them in the refrigerator (4-8°C). It is recommended to avoid frequent temperature changes and storage in the freezer.

Disposal

Test kits are completely contaminated after use and bacterial growth. As a result, they need to be autoclaved or burn in a furnace. If this is not possible, open the falcons under the laboratory hood and fill it with bleach liquid with a concentration of 5 to 10%. Let it sit overnight and then throw it away.